Technical Brief:

Why Use Serum-Free Medium?

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The use of serum-free medium and its related genera of animal-free and protein-free media has grown significantly in the last 15 years. This is particularly true in industrial applications where the use of serum presents a safety hazard as well as a source of unwanted contamination for the production of biopharmaceuticals[1]. Serum-free medium is prepared without the use of animal serum, but may contain serum constituents or substitutes thereof. Animal-free medium is similar to serum-free except that the components are derived from non-animal sources. Recombinant proteins replace native proteins and the nutrients are obtained from synthetic, plant or microbial sources. In contrast, protein-free medium is defined as devoid of protein, although few formulations of these media can be 100% protein-free without loss of function. A better definition would be low protein where minimal quantities of small mass proteins are employed.

The three types of media can be thought of as gradations of complexity. Serum-free media is the more complex composition designed for universal use in culturing mammalian cell lines. Animal-free and protein-free media formulations are less complex and more defined, but are limited to the cultivation of specific cell types. Thus, the selection of one medium over another will depend on the intended use. For most research applications, serum-free medium is the best choice as it provides broad-spectrum utility while giving good control of the composition. In contrast, in the manufacture of protein-based drugs potential contaminants and production costs play a more significant role in the selection of a more defined medium composition.

Serum-free medium has four basic advantages over serum containing medium. These include: 1] a simplified and better defined composition, 2] a reduced degree of contaminants, 3] elimination of a potential source of infectious agents, and 4] lower cost[2].

Simplified and Defined Composition

Serum is an ill defined component of medium. Typically used at 5% to 10% v/v, serum provides a range of factors which have proved necessary for the cultivation of mammalian and insect cells. Serum however can be derived from different sources and the composition of the serum can vary greatly. Advances in the manufacture and processing of serum has reduced this variability, but many researchers continue to find it necessary to qualify new lots of serum by comparing the growth of their cell lines in the new serum relative to a current lot. With a typical acceptance criteria of ±20%, the variance of whatever physiological function is being measured can be quite high. This leads to poor reproducibility of results between and within laboratories and stories abound of irreproducible results which can be directly attributed to the use of serum as a media component. A quantitative understanding of cellular physiology cannot be obtained with such ill-defined and variable conditions. Serum-free medium on the other hand is a more defined medium. While composed of many constituents, the composition is known and the level of each component precisely defined. Therefore, the variance seen with serum containing medium is eliminated giving a more controlled environment in which cells can be grown.

Reduced Range and Level of Contaminants

Medium prepared with serum at 10% has a protein concentration of 6,200 to 10,000 mg/L. A typical recombinant protein produced in mammalian cells is anywhere from a few mg/L to 1,000 mg/L. For a native protein, the level of accumulation can be even lower. The serum proteins thus become a major contaminant of any crude supernatant in which the target protein accumulates. Further, if the target protein is functionally, biochemically or physically related to a serum protein, then it may prove difficult to separate the target protein from the serum protein. This could be disastrous for the production of a protein-based drug, but equally troubling to the researcher trying to study the

functional and physical properties of the target protein. The protein concentration of serum-free media on the other hand is between 50 mg/L and 1,000 mg/L. Unlike serum, the composition is known and typically three proteins, albumin, transferin and insulin, comprise 80% to 90% of the proteins present. Consequently, the relative level of the target protein is significantly higher and with fewer contaminants. Thus, the target protein is easier to purify in fewer steps which gives higher recovery values.

Drug discovery, physiological, or gene expression studies are simpler to perform with serum-free medium. Serum components are known to bind, degrade or otherwise interact with chemicals added to the medium. Complex associations are possible between the serum, the added effector and the cells. Thus, the effect elicited by the added chemical can be altered or eliminated by the serum factors. Serum-free medium by contrast has fewer possible interferents and where an interaction is observed, more readily controlled.

Elimination of Potential Source of Infectious Agents

Viral, bacterial and fungal contamination of serum has for some years been a concern by manufacturers of biopharmaceuticals. This has been a driving force behind the adoption of serum-free, animal-free and protein-free media formulations in the manufacturing process. More recently, transmissible spongiform encephalophathies described in animal-derived materials has lead to the elimination of any animal product for the manufacture of a pharmaceutical product. In the research setting, where more fastidious cell lines are employed, the complete elimination of animal-derived components is both impractical and unusable. Recombinant forms of some key components remain expensive and in some cases not available. Many of the commercially available animal-free and protein-free media were designed for specific cell types and will not support the growth of a wide range of cell types. Because the materials used for the production of serum-free media are for the most part highly purified, the risk of contaminating a culture with adventitious agents is greatly reduced or eliminated altogether. For non-pharmaceutical applications, serum-free medium remains a good balance between safety and efficacy.

Lower Cost and Availability

Serum can cost between $7 and $50 per liter of medium depending on the type and percentage of serum used. Fetal calf serum, the most commonly used source of serum, is also the most costly, with current prices (August 2002) averaging $456/L of serum. Therefore, serum can contribute significantly to the cost of the medium. Serum-free medium by contrast averages about $120/L with a range from $26/L for a DMEM derivative to $692/L for highly specialized bone marrow medium. Most of the serum-free media are supplied ready-to-use, so are available without the need of preparing a multi-component reagent.

Disadvantages

The use of serum-free medium is routine for many cell types and many formulations are available in the literature or through a number of vendors. Nevertheless, there are a few drawbacks that should be considered when using serum-free medium. First, an investment in time is required to adopt a particular cell line to serum-free medium. The cells will have to be weaned from serum slowly. Moreover, some cell lines may require the addition of growth factors specific to that cell type to overcome a deficiency in the particular medium employed. It is advisable to begin with a serum-free medium which has a source of growth factors, such as pituitary extract, which can be incrementally removed if necessary.

The second limitation is that the low protein concentration of serum-free medium while an advantage for reducing potential sources of contamination removes proteins which play a role in shear protection and attachment to growth substratum. The BSA present in serum protects cells grown in suspension from shear damage. The addition of Pluronic F68 or polyethylene glycol may be needed in place of BSA. BSA also provides other transport functions and the replacement of native sources of albumin with recombinant sources is not straightforward. Alternative supplements may be needed. Many of these issues are resolved by the commercially available formulation and only become a concern when developing a custom medium.

Attachment-dependent cell lines require an extracellular matrix on the growth substratum. Serum provides some the components for this matrix. Therefore, when using serum-free medium the substratum (plastic dishes) should be pre-coated with a fibronectin, laminin or another suitable alternative such as FNC Coating Mixâ (a fibronectin/collagen mixture manufactured by AthenaES, Baltimore, MD), Pronectinâ (a synthetic fibronectin polymer manufactured by Sanyo Chemical Industries, Kyoto, Japan) or Matrigel.

Summary

Serum-free medium is an excellent alternative to standard serum-containing media for the cultivation of cells. It has several marked advantages which include better definition of the composition, reduced contamination by adventitious and infectious agents, and lower cost. Further, the commercial availability of many variations of serum-free media has made it easy to obtain and employ. Consequently, the use of serum-free medium has now become a routinely used reagent in many laboratories for the culture of a wide variety of cell types.

About the Author

Sheldon E. Broedel, Jr., Ph.D. serves as the Chief Science Officer at AthenaESÔ. He has over 23 years of research experience, 15 of which were spend in the biotechnology industry. Dr. Broedel has broad expertise in a range of biology disciplines with over 20 products and patents to his credit. Currently, his research team is developing reagents designed to increase the production of recombinant proteins.

(Endnotes)

[1] Merten, O.-W. Safety issue of animal products used in serum-free medium. *In* Animal Sera, Animal Sera Derivatives and Substitutes Used in the Manufacture of Pharmaceuticals: Viral Safety and Regulatory Aspects. Dev. Biol. Stand. Brown F., Cartwright T., Horaud, F. and Spieser, J.M. eds., Basel, Karger, 1999, Vol. 99:167-180.

[2] Froud, S. J. The development, benefits and disadvantages of serum-free media. *Ibid.* pp. 157-166.